Common Causes of Limb Abnormalities: Sequence Analysis and Exon-Level Deletion/Duplication Testing of 14 Genes

Panel Gene List:
ESCO2, HDAC8, LMBR1 including ZRS regulatory region*, NIPBL, NSDHL, RAD21, SALL1, SALL4, SHH, SMC1A, SMC3, TBX5, TP63 and WNT3*
**deletion/duplication testing not included.*

Clinical Features:
Limb abnormalities are a broad category of genetically heterogeneous syndromic and non-syndromic skeletal disorders. Syndromic limb abnormalities include conditions such as Cornelia de Lange syndrome (HDAC8, NIPBL, RAD21, SMC1A and SMC3 genes)[2,10], Robert's syndrome (ESCO2 gene)[5], CHILD syndrome (NSDHL gene)[3], Townes-Brocks syndrome (SALL1 gene)[7], Duane-radial ray syndrome (SALL4 gene)[6], Holt-Oram syndrome (TBX5 gene)[11], Tetra-amelia syndrome (WNT3 gene)[12,13], and multiple syndromes associated with pathogenic variants in the TP63 gene[14]. In addition to the limb abnormalities observed in these syndromes, affected individuals may or may not have additional clinical features such as pre- and postnatal growth restriction, microcephaly, distinctive facial features, and central nervous system abnormalities including intellectual disability[2,5,12,14]. Other organ manifestations vary between syndromes and can affect any combination of body systems including the cardiovascular and renal systems[2,3,5-7,11,12,14]. Non-syndromic limb abnormalities can be caused by pathogenic variants in the SHH gene as well as in its' regulatory element, the ZRS region of the LMBR1 gene[9].

Pathogenic variants in these genes cause a variety of limb malformations that range from complete absence of all four limbs to mild phalangeal abnormalities. Pre-axial polydactyly and triphalangeal thumbs are common to a number of syndromes, whereas split-hand/foot malformation is unique to TP63 variants.

Inheritance Pattern/Genetics:
Autosomal dominant, autosomal recessive and X-linked inheritance.

Test Methods:
Using genomic DNA obtained from the submitted specimen, the coding exons and flanking splice junctions of the genes on this panel are enriched using a proprietary targeted capture method developed by GeneDx. These targeted regions are simultaneously by massively parallel (NextGen) sequencing on an Illumina platform with paired-end reads. Bidirectional

sequence is assembled, aligned to reference gene sequences based on human genome build GRCh37/UCSC hg19, and analyzed for sequence variants. Capillary sequencing is used to confirm all potentially pathogenic variants and to obtain sequence for regions where fewer than 15 reads are achieved by NextGen sequencing. Concurrent deletion/duplication testing is performed for the genes in the panel (except LMBR1, the ZRS regulatory region of SHH, and WNT3) using exon-level oligo array CGH (ExonArrayDx). Data analysis is performed using gene-specific filtering. Probe sequences and locations are based on human genome build GRCh37/UCSC hg19. The array is designed to detect most intragenic deletions and duplications. Confirmation of copy number changes is performed by MLPA, qPCR, or repeat array CHG analysis. Sequence and array CGH array CGH alterations are reported according to the Human Genome Variation Society (HGVS) or International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively. Benign and likely benign variants, if present, are not included in this report but are available upon request.

Test Sensitivity:

Limb abnormalities are a genetically heterogeneous group of disorders with a wide variant spectrum. The clinical sensitivity of sequence and deletion/duplication analysis of the 14 genes included in this panel depends on the clinical phenotype of the patient. Additional sensitivity information is available in the table below.

The technical sensitivity of the sequencing test is estimated to greater than 99%. It will not detect deletions, insertions, or rearrangements greater than or equal to ten base pairs. The deletion/duplication testing can detect deletions or duplications encompassing one or more exons, including variants as small as 150-300 base pairs. Note that small sections of a few individual genes have inherent sequence properties that yield suboptimal data and variants in those regions may not be identified.

Clinical Sensitivity of Genes Associated with Common Limb Abnormalities

Disorder(s)	Gene	Inh.	Diagnostic Yield for Disorder
Roberts syndrome; SC phocomelia syndrome	*ESCO2*	AR	100% for Roberts syndrome[5]
Cornelia de Lange syndrome 5	*HDAC8*	XLD	~4% of CdLS[2]
Acheiropody (AR); Hypoplastic or aplastic tibia with polydactyly; Laurin-Sandrow syndrome; Polydactyly, preaxial type II; Syndactyly, type IV; Triphalangeal thumb, type I; Triphalangeal thumb-polysyndactyly syndrome	*LMBR1* including ZRS regulatory region of SHH*	AD AR	Unknown
Cornelia de Lange syndrome 1	*NIPBL*	AD	~60% of CdLS[2]

CHILD syndrome; CK syndrome	*NSDHL*	XLD XLR	Unknown, but expected high for CHILD syndrome[1,3,8]
Cornelia de Lange syndrome 4	*RAD21*	AD	<1% CdLS[2]
Townes-Brocks syndrome; Townes-Brocks branchiootorenal-like syndrome	*SALL1*	AD	~75% for Townes-Brocks syndrome[7]
Duane-radial ray syndrome; IVIC syndrome	*SALL4*	AD	~90-95% for DDRS[6]
Holoprosencephaly 3; Microphthalmia with coloboma 5; Schizencephaly; Single median maxillary central incisor; see LMBR1 for other disorders related to pathogenic variants in ZRS regulatory region of SHH	*SHH*	AD	Unknown
Cornelia de Lange syndrome 2	*SMC1A*	XLD	~5% of CdLS[2]
Cornelia de Lange syndrome 3	*SMC3*	AD	1-2% of CdLS[2]
Holt-Oram syndrome	*TBX5*	AD	>70% for HOS[11]
ADULT syndrome; Ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome 3; Hay-Wells syndrome; Limb-mammary syndrome; Orofacial cleft 8; Rapp-Hodgkin syndrome; Split-hand/foot malformation 4	*TP63*	AD	~98% for EEC. ~10% for SHFM[14]
Tetra-amelia syndrome	*WNT3**	AR	Unknown[13]

* This panel does not include deletion/duplication testing of LMBR1, including the ZRS regulatory region, and WNT3.

Abbreviations for Inheritance (Inh):
AD – Autosomal dominant
XLD – X-linked dominant
AR – Autosomal recessive
XLR – X-linked recessive

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